APPENDIX A - Protocol

STILLMEADOW
INCORPORATED

PROTOCOL For STUDY 24071-20

Test Substance:	BEHR Antibacterial Paint, #3190
Study Title:	ACUTE DERMAL TOXICITY In RATS
Guideline:	OCSPP 870.1200
Test Facility:	STILLMEADOW, Inc. 12852 Park One Drive Sugar Land, TX 77478

Approved: Vincent A. Murphy — 04 Nov 20
Vincent A. Murphy, PhD, DABT
Study Director, STILLMEADOW, Inc.
Date

Approved: [signature] — 02 Nov 20
Management, STILLMEADOW, Inc.
Date

Reviewed: [signature] — 02 Nov 20
Kristina Rodrigue, RQAP-GLP
Quality Assurance Director, STILLMEADOW, Inc.
Date

Sponsor: BEHR Paint Company
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Approved: [signature] — 11/03/2020
John Gilbert
Chief R&D Officer
Date

A. GENERAL

1.	Study Title:	Acute Dermal Toxicity in Rats
2.	Purpose:	To assess systemic toxicity and relative skin irritancy of test substance when applied to the skin of rats.
3.	Method Guidelines:	This study will be conducted according to US OCSPP 870.1200.
4.	Regulatory Compliance:	This study will be conducted in compliance with Good Laboratory Practice (GLP) standards: 1. EPA FIFRA 40 CFR 160 In the event of a regulatory inspection, Regulatory Inspectors will be provided with all study documentation requested. Sponsor will be notified of inspection of their study. All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOP).
5.	Quality Assurance:	The Quality Assurance Unit (QAU) will review the protocol. Study information will be entered into the master schedule. In-progress inspection(s) will be performed to ensure integrity of the study. Any deviations from SOP, protocol or GLP standards will be reported to Study Director and Management. Raw data and report will be audited, and a statement prepared and signed which will specify dates inspections were made and findings reported to Management and Study Director.
6.	Test Substance:	BEHR Antibacterial Paint, #3190. Test substance identification should include name, lot/batch number and purity. Sponsor should also provide information regarding safety, storage conditions and disposal. Sponsor assumes responsibility for purity, stability, identity, synthesis methods and location of documentation.
7.	Proposed Schedule:	Testing should begin after test substance receipt, authorization to conduct study and study initiation. Proposed Experimental Start & End: 05 Nov 20 - 19 Nov 20 Study will be extended if extra dose levels are required.
8.	Study Director:	Vincent A. Murphy, PhD, DABT
9.	Experimental Summary:	Test substance will be applied to intact skin of albino rats and maintained in contact with skin for 24 hours. Animals will be observed several times on the day of dosing and daily after for mortality and pharmacologic and/or toxicologic signs, for 14 days. If sufficient number of dose levels are tested, LD_{50} with slope and 95% confidence limits will be calculated.
10.	Protocol Amendments:	Any protocol alteration will be justified, approved by Study Director, and recorded in writing.
11.	Sponsor Audits:	Sponsor may send an authorized Representative to inspect test system and/or data on STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a.	Species/Strain/Source:	Albino rat / Sprague-Dawley / Texas Animal Specialties; Humble, TX (or other suitable source)
b.	Species Justification:	The rat is conventionally used in toxicity tests to provide information on which human hazard can be judged, and is one species preferred by regulatory agencies.
c.	Quantity & Sex:	5 male, 5 female (nulliparous & non-pregnant) for initial dose level; additional animals may be required (see B.3.g.)

B.	1.	d.	Age/Weights on Day 0:	8 - 12 week / Male: 225 - 375 g, Female: 175 - 250 g Weight variation should not exceed ± 20% of mean for each sex
		e.	Identification:	Ear punch
		f.	Acclimation & Health Status:	Animals will be acclimated for at least 5 days prior to dosing. Normal weight gain, appearance and behavior will be factors used to select healthy naive animals for testing.
		g.	Randomization:	No formal randomization procedure will be required.
	2.	Animal Husbandry		
		a.	No./Cage & Cage Type:	Individually housed during study in polycarbonate box with bedding
		b.	Enrichment:	Provided to each animal during study
		c.	Food:	Teklad Global Diets® #2018, or equivalent, available ad libitum; analyzed by manufacturer for nutritional content
		d.	Water:	Tap water, available ad libitum (automatic system); municipal water supply analyzed by TCEQ Water Utilities Division
		e.	Contaminants:	There are no known contaminants in feed or water available to laboratory animals that would be expected to interfere with this study.
		f.	Environment:	Target temperature: 22° ± 3°C Target relative humidity: 30 - 70% 12-hr light/12-hr dark cycle (regulated automatically) Room ventilation: at least 10 air changes per hour
	3.	Test Substance Administration		
		a.	Animal Preparation:	At least 24 hours before dosing, each animal will be clipped free of hair along the back of the trunk to expose not less than 10% of total body surface area. Care will be taken to avoid abrading skin. Animals with exposure areas free from pre-existing skin irritation or defects will be selected for testing. Animals will be clipped as needed throughout study.
		b.	Reason for Route of Administration:	Dermal contact is a potential route of human exposure.
		c.	Animal Group Assignment:	Animals will be randomly selected for dose groups so individual body weights not exceed ± 20% of mean weight for each sex.
		d.	Test Substance Preparation:	Aerosol substance will be discharged into a container and administered as a liquid. Solid test substance will be pulverized if necessary.
		e.	Test Substance Application:	All animals will receive a single administration of test substance on Day 0 based on body weight on that day. Test substance will be applied evenly to exposure area to make as thin and uniform a layer as possible under surgical gauze. Liquid test substance will be applied as received. Solid test substance will be moistened with sufficient quantity of DI water or saline to make a thick paste before application; if aqueous vehicle is not appropriate, corn oil is preferred. Other acceptable vehicles include gum arabic, ethanol plus water, glycerol, propylene glycol, CMC, PEG, and vegetable/mineral oil. Justification for vehicle other than water or saline must be supplied. Application area will be covered with appropriate size gauze patch (~2 x 4 in. or larger if necessary) and secured, if needed, with non-irritating adhesive tape. The trunk of each animal will be wrapped with veterinary flexible cohesive bandage (Equi-Flex, VetFlex, or other suitable material).

B. 3. f. Test Substance Removal: Wrappings will be removed after a 24-hour exposure period. If necessary, the skin may be dry-wiped or washed with room temperature tap water to remove as much residual substance as possible.

g. Dose Level: **5050 mg/kg dose level** will be tested on five rats per sex. If no mortality occurs among these animals, no further testing is necessary. If mortality exceeds 40% in either or both sexes at the level tested, then an additional dose level of 2020 mg/kg will be tested for appropriate sex so LD_{50} can be determined. There will be at least five animals (5 male or 5 female) per dose level. If both sexes are tested at a given dose level, the group will consist of an equal number of males and females. If one sex is markedly more sensitive, testing of other sex may be dispensed with.

4. Observations

a. Clinical Signs: Observations for mortality and pharmacologic and/or toxicologic effects will be made three times on day of dosing, and at least once daily after for 14 days. Nature, onset, severity and duration of all gross or visible pharmacologic/toxicologic signs will be recorded. Observations include: evaluation of skin, fur, eyes, mucous membranes; respiratory and circulatory effects; autonomic effects such as salivation, lacrimation, excessive urination, diarrhea; central nervous system effects including tremors and convulsions; changes in activity, gait, posture; reactivity to handling or sensory stimuli; altered strength and stereotypies or bizarre behavior (e.g., self mutilation, walking backwards). Animals with significant signs of pain/ distress will be observed more often and, if need, given sufficient concentration of suitable analgesic by subcutaneous or IM injection; analgesic will be readministered, if need, at appropriate frequency.

b. Body Weights: Body weights will be recorded on day of dosing (Day 0) and weekly after (Days 7 and 14), or at time of discovery after death.

c. Dermal Irritation: After removal of wrappings on Day 1 (typically ~1 hour past removal), exposure area of each animal will be examined for evidence of dermal irritation (Legend A), and again on Days 4, 7, 11 and 14. At the first observation, if any, of excessive irritation causing/expected to cause pain/ distress, a sufficient concentration of suitable analgesic will be administered by subcutaneous or IM injection. Until irritation lessens, analgesic will be readministered at an appropriate frequency.

d. Animal Sacrifice: Animals with signs of severe pain/distress considered irreversible will be humanely euthanized, per Study Director decision. All animals surviving to study termination will be euthanized by CO_2 overdose.

e. Necropsy: Gross necropsy will be conducted on each animal at termination of the study or at time of discovery after death, and results recorded (generally only abnormalities if any, or NOA if none). Gross necropsy shall include gross observations of external surfaces; all orifices; and thoracic, abdominal and pelvic cavities.

5. Evaluation of Results: Unless only a single dose level is tested, LD_{50} with (if applicable) slope and 95% confidence limits will be calculated for males, females, and sexes combined by method of Rosiello, Essignmann & Wogan: Rapid and Accurate Determination of Median Lethal Dose and its Error with a Small Computer, Journal Toxic Environ Health, 797 - 809, 1977, or other appropriate method. Toxicity Category may be assigned from LD_{50}.

6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. Test substance container(s) will be weighed when received at this facility, and all test substance use recorded. Test substance and substance dosing solutions will be stored in original containers or equivalent, or in capped glass containers.

7. Unused Test Substance Disposal: Unused test substance will be disposed of at Sponsor's expense after study termination.

B. 8. Safety Precautions:

General safety precautions required by laboratory SOP will be followed. Sponsor will supply basic toxicity data on test substance to be used; however, since toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. Sponsor or Representative shall be notified of any exposure requiring physician's exam or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained at STILLMEADOW, Inc. during the study, and archived upon study termination:

a. Protocol & protocol amendments (if any)
b. Final report & amendments (if any)
c. Study correspondence
d. Animal receipt/acclimation data
e. Test substance receipt, identification supplied by Sponsor, preparation, administration, disposition
f. Test animal information: species, strain, sex, source, number
g. Body weight data
h. Daily observation data for pharmacologic &/or toxicologic signs
i. Mortality data & gross necropsy findings
j. LD_{50} & calculations (if any) of slope with 95% confidence limits
k. Scores for dermal irritation
l. Other pertinent data

2. **Data Storage:**

All raw data, originals of protocol, final report, any amendment(s) and a test substance sample will be archived at STILLMEADOW, Inc. for 15 years.

3. Data Reporting:

Final report will include following data as described in GLP standards:

a. Statement from QAU
b. GLP Compliance Statement & signature of Study Director
c. Names of scientific personnel involved in study
d. Dates of study initiation & termination
e. Identification, label information, description, preparation, storage of test substance
f. All pertinent animal data & husbandry, dosing information, observation methods
g. Description of test procedures
h. LD_{50} &, if calculated, slope with 95% confidence limits for males, females, & sexes combined
i. Individual body weights
j. Observations on nature, onset, severity & duration of all gross or visible pharmacologic &/or toxicologic signs; nonroutine findings will be addressed in a discussion section
k. Individual mortality data & gross necropsy findings
l. Dermal irritation scores (if any)
m. Copy of this protocol; deviations (if any) & impact on study

4. Report Generation:

A final report will be generated after termination of in-life portion of the study; a draft report may first be issued for Sponsor approval.

LEGEND A - Primary Dermal Irritation Scoring Scale (Draize Technique)

Erythema Formation	Score	Edema Formation	Score
None	0	None	0
Very slight (barely perceptible)	1	Very slight (barely perceptible)	1
Well-defined	2	Slight (edges well-defined)	2
Moderate	3	Moderate (raised ~1 mm)	3
Severe (beet redness)	4	Severe (raised >1 mm & beyond test area)	4

Other observations may be noted, for example: necrosis, eschar, etc.